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Chapter 46

Molecular Weight Determinations

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Summary

General Uses

- Determination of absolute and apparent molecular weight distribution (MWD) for polymers and biopolymers
- Determination of polymer concentration in a matrix
- Determination of copolymer and blend composition (multidetector methods)
- Determination of branching, in conjunction with low-angle laser light scattering or viscometry
- Sample cleanup
- Chromatographic cross-fractionation of copolymers (in conjunction with precipitation or adsorption chromatography)
- Separation of small molecules
- Preparative size exclusion chromatography (SEC) for isolating relatively large quantities of particular components

Common Applications

- Determination of MWD of organic and aqueous-soluble polymers
- Establishing molecular weight/property relationships
- Quality control
- Sample cleanup for determination of additives or oligomer distributions

Samples

State

Solid or liquid polymer samples can be analyzed.

Amount

For polydisperse samples, 25 to 100 mg is desirable. For monodisperse samples, 1 to 20 mg is desirable.

Preparation

Sample is dissolved in SEC eluent and filtered before analysis. Care is taken not to fractionate the sample on the filter membrane.

Analysis Time

Sample preparation consists of weighing the sample, adding a specific volume of solvent, and using light agitation to achieve dissolution. Dissolution can be the rate-limiting step for SEC analysis time, requiring on the order of 0.5 to 24 hr or more, depending on the sample. Because alteration of the property to be measured may occur, devices that induce high shear on the polymer sample (such as ultrasonic baths) should not be used to expedite sample dissolution. Additionally, crystalline polymers often require heating to achieve dissolution, but one should determine whether heating changes the sample MWD before applying heat.

A chromatogram can be obtained in 10 to 60 min depending on the number of columns used and the eluent flow rate. Data analysis (with a computer) requires only a few minutes per chromatogram.

Calibration with narrow MWD standards requires 1 to 2 hr depending on the number of standards used to establish the calibration. Typically, up to four narrow fraction standards can be analyzed in a single injection, provided there are sufficient molecular weight differences to achieve baseline resolution.

Limitations

General

- Only apparent molecular weights are determined unless molecular weight standards exist of the same composition and topology as the unknown, LALLS or viscometry is used, or the Mark–Houwink parameters of standards and sample are known.
- Suitable or compatible solvents must be found for characterization of difficult-to-dissolve polymers (such as nylon).
- SEC is an inherently low-resolution technique, but this is typically not a real limitation for broad-MWD synthetic polymers.

Accuracy

For samples where absolute molecular weight calibration is possible, we can readily achieve accuracy within $\pm 5\%$.

Complementary or Related Techniques

For Molecular Weight Distributions

- Thermal field flow fractionation: For a given composition, polymer molecules are separated according to differences in the ordinary (Fick's) diffusion coefficient. Polymers of different composition are separated according to differences in the thermal diffusion coefficient.
- Mass spectrometry: Matrix-assisted laser desorption time-of-flight mass spectrometry has been applied to synthetic polymers and proteins of relatively low molecular weight (less than 150,000 Da).
- Ultracentrifugation: This tedious, older technique relies on the measurement of sedimentation coefficients, which can be used to calculate molecular weights. Although still used sparingly, this technique has been made obsolete by more current polymer characterization techniques.

For Molecular Weight Averages Only

- Static low-angle laser light scattering for absolute weight-average molecular weight
- Colligative properties for absolute number-average molecular weight (such as vapor phase osmometry)
- End-group analysis for absolute number-average molecular weight (such as titrations or nuclear magnetic resonance)
- Solution viscosity for measurement of viscosity-average molecular weight, provided one knows or can measure the Mark-Houwink coefficients
- Quasielastic light scattering (or photon correlation spectroscopy) to determine diffusion coefficients of polymers, which are related to polymer molecular weight

Introduction

Size exclusion chromatography (SEC) is an extremely important technique for characterizing macromolecules. SEC is a high-performance liquid chromatography (HPLC) technique in which molecules are separated according to differences in hydrodynamic volume. This separation mechanism is made possible by the packing material in the column. The packing material is made of porous (rigid or semi-rigid) spherical particles (3 to 20 μm). The retention in SEC is governed by the partitioning (or exchange) of the macromolecular solute molecules between the mobile phase (the eluent flowing through the column) and the stagnant liquid phase that occupies the interior of the pores. The range of macromolecular sizes that can be separated with a given column depends on the size (or size distribution) of the pores. The resulting chromatogram in an SEC experiment thus represents a molecular size distribution.

The relationship between molecular size and molecular weight depends on the conformation of the dissolved solute molecules. However, for any solute conformation (such as random coil, rigid rod, or hard sphere), the molecular size increases with molecular weight. The rate at which the molecular size increases with molecular weight varies for the different possible solute conformations. Most synthetic polymer samples can be categorized as random coil solutes. As such, the relationship between molecular size (radius of gyration, R_g) and molecular weight is $R_g \propto M^a$, where the exponent is a constant dependent on the solute composition, the temperature, and the solvent. Given that the molecular size is related to the molecular weight of the polymer, the molecular size distribution obtained in the SEC experiment can be converted into a molecular weight distribution (MWD) if we can establish a molecular weight versus retention volume calibration.

How It Works

The diagram in Fig. 46.1 illustrates the sequence of events that occur during an SEC experiment. Large solutes elute from the column first; smaller solutes elute from the column later. It illustrates the analysis of a hypothetical mixture of large and small polymer solutes by SEC. The first frame in Fig. 46.1 shows the injection of the mixture as a narrow band at the head of the column. In the second frame, the development of size separation is illustrated. In this frame, the larger polymer solutes are clearly migrating through the column at a faster rate than the smaller solutes. The larger solutes are more excluded from the pores in the packing, and thus spend more time in the mobile phase than in the stagnant mobile phase contained within the pores. The smaller solutes can enter more of the pores containing the stagnant mobile phase. As a result, the smaller solutes spend more time in the column. In the third frame, the larger molecules elute from the column and are detected by a suitable detector, and in the last frame, the small molecules elute.

The sequence of events illustrated in Fig. 46.1 serves only to describe the mechanism of separation in SEC. Real samples of synthetic polymers usually contain a broad distribution of molecular sizes that cannot be separated into individual SEC peaks. Rather, an SEC chromatogram of a given polymer sample is typically a broad peak, with the earlier-eluting species representing the high-molecular-weight species and the late-eluting species representing the low-molecular-weight species.

In SEC, retention depends on the continuous exchange of solute molecules between the mobile phase and the stagnant mobile phase within the pores of the packing. This exchange is an equilibrium, so entropy-controlled processes and enthalpic processes such as adsorption are undesirable in SEC. Thus, the SEC retention volume (V_r) is expressed as shown in Eq. (46.1).

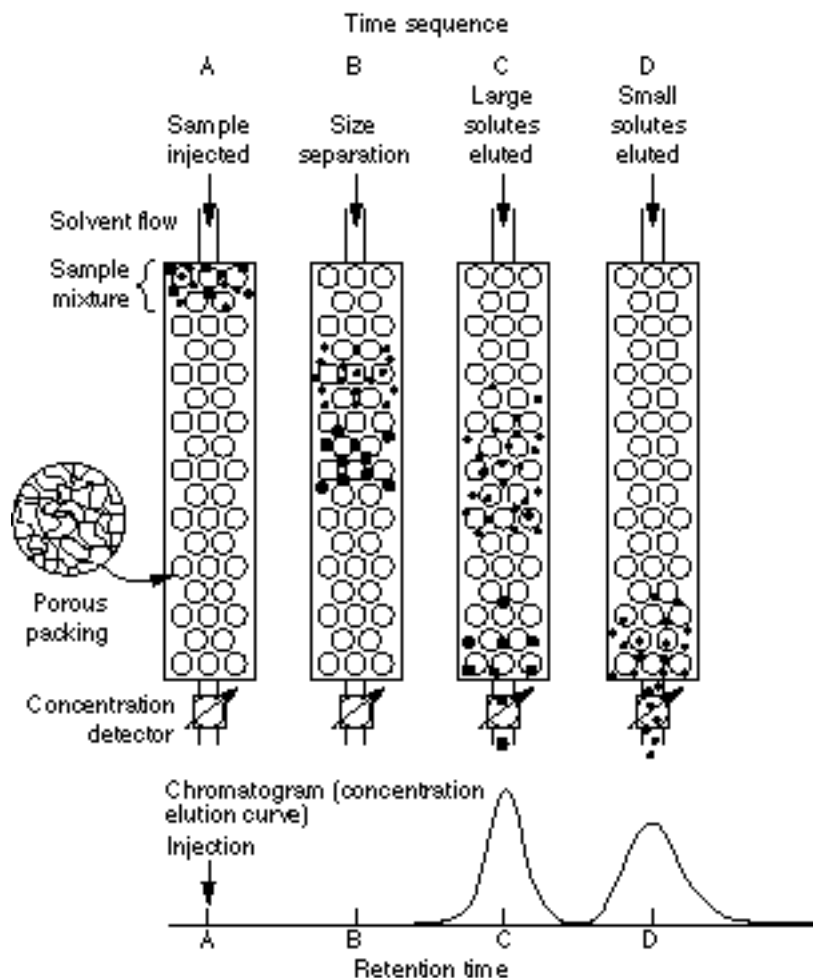
$$V_r = V_o + V_p K_{\text{SEC}} + V_s K_{\text{LC}} \quad (46.1)$$

where V_o = the exclusion volume or interstitial volume between the packing particles (defined in Fig. 46.2), V_p = the pore volume (defined in Fig. 46.2), V_s = the stationary phase volume, K_{SEC} = the SEC solute distribution coefficient, and K_{LC} = the liquid chromatography solute distribution coefficient.

Because ideal SEC retention is governed only by entropic contributions, the column packing material/eluent combination is chosen such that K_{LC} is minimized or ideally $K_{\text{LC}} = 0$.

The dependence of V_r on solute molecular weight represents the SEC calibration curve. An example calibration curve, generated from an injection of four narrow-fraction molecular weight standards, is shown in Fig. 46.2. In this example, the highest-molecular-weight species is entirely excluded from the pores in the packing, so its retention volume is equal to the interstitial or exclusion volume, in which case K_{SEC} is zero. The lowest-molecular-weight standard permeates all of the pores within the SEC column, so its retention volume equals the sum of the interstitial volume and the pore

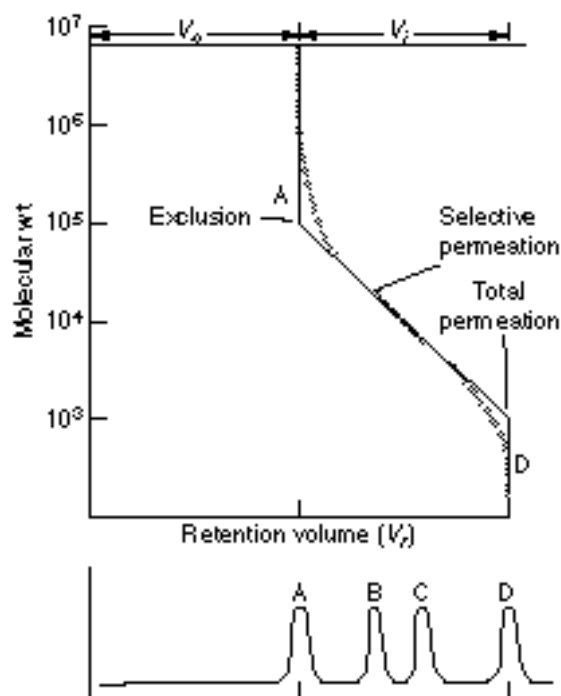
Figure 46.1 Illustrative description of separation in SEC. (From *Introduction to Modern Liquid Chromatography*, 2nd edition by L. Snyder and J. J. Kirkland, © 1979 by John Wiley & Sons, Inc. Reprinted by permission of John Wiley & Sons, Inc.)



volume. This volume is called the total volume or total permeation limit. The value of K_{SEC} for species eluting at the total permeation limit is 1. The two intermediate-molecular-weight standards can permeate the pores to some extent, so they are separated according to their respective hydrodynamic volumes. The value of K_{SEC} for peaks eluting in this region is $0 < K_{\text{SEC}} < 1$.

The linear region in Fig. 46.2 defines the useful region for SEC separation of macromolecules. The molecular weight range covered by the linear region of the SEC calibration curve depends on the size of the pores in the packing material. For single pore size columns, the useful region for SEC covers approximately 2 decades in molecular weight. (Gels with pore sizes ranging from 106 to 50 Å are typically available in a wide variety of compositions.) Most synthetic polymer samples have broad MWDs that span more than 2 decades in molecular weight. Individual pore size columns can be coupled to provide the required separation range, or mixed-bed columns (columns containing a mixture of pore sizes) can be used. Mixed-bed columns are designed to provide several orders of magnitude (4 to 5) in molecular weight separation range. The choice of column depends on the sample composition, solvent options, and molecular weight range. The goals are to have ideal SEC retention (that is, separation by size

Figure 46.2 Log molecular weight versus retention volume plot for a typical SEC experiment with four narrow-fraction molecular weight standards. (From *Introduction to Modern Liquid Chromatography*, 2nd edition by L. Snyder and J. J. Kirkland, © 1979 by John Wiley & Sons, Inc. Reprinted by permission of John Wiley & Sons, Inc.)



only) and optimal resolution. Resolution in SEC depends on the slope (and column dispersion) of the linear calibration region. The column should be chosen such that the linear range encompasses the entire MWD of the sample with the smallest (absolute value) slope available. A detailed discussion of resolution in SEC and its impact on molecular weight accuracy is beyond the scope of this chapter. The interested reader is encouraged to consult the Suggested Readings.

What It Does

Size exclusion chromatography is widely used for determination of polymer molecular weight and MWD. For polymer characterization, molecular weight and MWD are important because they can have significant impact on physical properties. Because SEC is useful for determination of molecular weight and MWD, it is an important technique for aiding in the establishment of structure-property relationships for polymers. Once molecular weight/property relationships are established, SEC can be used in quality control fashion to ensure the production of polymers having desirable physical properties. SEC can also be used to aid in optimization of process conditions for production of materials with desirable molecular weight. SEC may also be used for comparing the MWD of samples that may perform differently in end-use applications.

Synthetic polymers differ from small molecules in that they cannot be characterized by a single molecular weight. In a synthetic polymerization, a distribution of chain lengths (that is, molecular

weights) is produced. This distribution can be described by any number of molecular weight averages. The most common molecular weight averages used in establishing molecular weight/property relationships are the number-average (M_n), weight-average (M_w), and z-average (M_z) molecular weights. These averages are defined by the following expressions:

$$M_n = \frac{\sum_i N_i M_i}{\sum_i N_i} = \frac{\sum_i W_i}{\sum_i W_i / M_i} = \frac{\sum_i h_i}{\sum_i h_i / M_i} \quad (46.2)$$

$$M_w = \frac{\sum_i N_i M_i^2}{\sum_i N_i M_i} = \frac{\sum_i W_i M_i}{\sum_i W_i} = \frac{\sum_i h_i M_i}{\sum_i h_i} \quad (46.3)$$

$$M_z = \frac{\sum_i N_i M_i^3}{\sum_i N_i M_i^2} = \frac{\sum_i W_i M_i^2}{\sum_i W_i M_i} = \frac{\sum_i h_i M_i^2}{\sum_i h_i M_i} \quad (46.4)$$

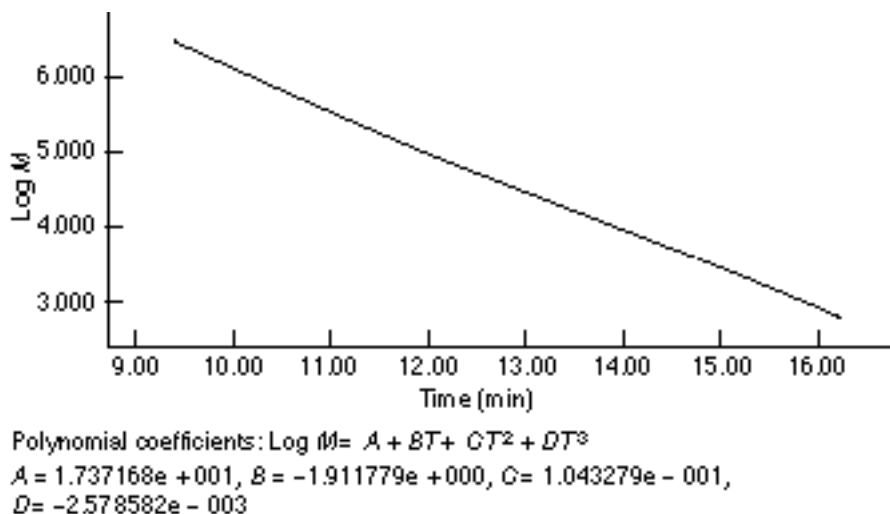
Here N_i and W_i are the number and weight, respectively, of molecules having molecular weight M_i . The subscript i is an index representing all molecular weights present in the ensemble of chains. Note that each of the above equations contains three representations of the particular molecular weight average. The third representation in each case (farthest right) defines how one obtains these averages from SEC chromatograms. h_i is the height (from baseline) of the SEC curve at the i th elution increment and M_i is the molecular weight of species eluting at this increment. M_i is obtained via calibration with appropriate standards.

Conversion of the retention volume axis in SEC to a molecular weight axis (that is, calibration) can be accomplished in a number of ways including peak position, universal calibration, broad standard (integral and linear), and determination of actual molecular weight. In the peak position method, a series of well-characterized, narrow-fraction molecular weight standards of known peak molecular weight (M_p) are injected onto the SEC system and the retention volumes determined. A plot of $\log M_p$ versus retention volume is constructed as shown in Fig. 46.3. Depending on the type of SEC columns used, the calibration data points can be fit with either a linear or a third-order polynomial function. For determination of absolute molecular weights, the peak position calibration is of limited utility because only a few commercially available standards exist, and in order for the molecular weight calibration to be valid, the composition and topology of the standards and unknown must be the same.

We could prepare and characterize calibration standards via preparative fractionation schemes and absolute molecular weight techniques (such as LALLS), but these efforts can be costly and time-consuming. In some instances, the accuracy of the calibration is not important because the researcher may be interested only in comparing two samples to determine whether there is a relative difference in molecular weight. In these cases, only apparent molecular weight information is obtained, and for comparison of sample MWDs, calibration may not even be necessary. However, in other cases, absolute molecular weight data may be necessary. In these cases, alternative calibration schemes exist.

One such scheme is the universal calibration principle shown in Fig. 46.4. The product of intrinsic viscosity, $[\eta]$, and molecular weight is proportional to hydrodynamic volume, and in ideal SEC, molecules are separated according to hydrodynamic volume. In 1967, Benoit showed that the calibration for polymers of different types can be merged into a single line when plotted as $\log [\eta]M$ versus retention volume, as opposed to the typical $\log M$ versus retention volume shown in Fig. 46.3. There are two ways to use the universal calibration principle to glean absolute molecular weight data. One way is to use an on-line viscometer to determine $[\eta]$ at each SEC retention increment. Well-characterized narrow-fraction molecular weight standards can be used to generate the universal calibration curve. The

Figure 46.3 SEC calibration curve for 17 narrow-fraction polystyrene molecular weight standards analyzed using two mixed-gel SEC columns and tetrahydrofuran as the mobile phase. The line is a third-order polynomial fit of the data points.



plot of $\log [h]M$ versus retention volume and the measured $[h]$ s for the sample allow one to calculate M at each retention volume increment.

Alternatively, one may combine the universal calibration principle and the Mark-Houwink equation to determine absolute molecular weights. From the universal calibration principle,

$$[h]_{i,A} M_{i,A} = [h]_{i,B} M_{i,B} \quad (46.5)$$

where the subscript i refers to the particular SEC retention time increment and the subscripts A and B refer to polymers of different composition. The Mark-Houwink equation is given below.

$$[h] = kM^a \quad (46.6)$$

where k and a are constants that depend on polymer composition, temperature, and solvent. Combining Eqs. (46.5) and (46.6) yields

$$k_A M_{i,A}^{1+a_A} = k_B M_{i,B}^{1+a_B} \quad (46.7)$$

Rearrangement of Eq. (46.7) gives

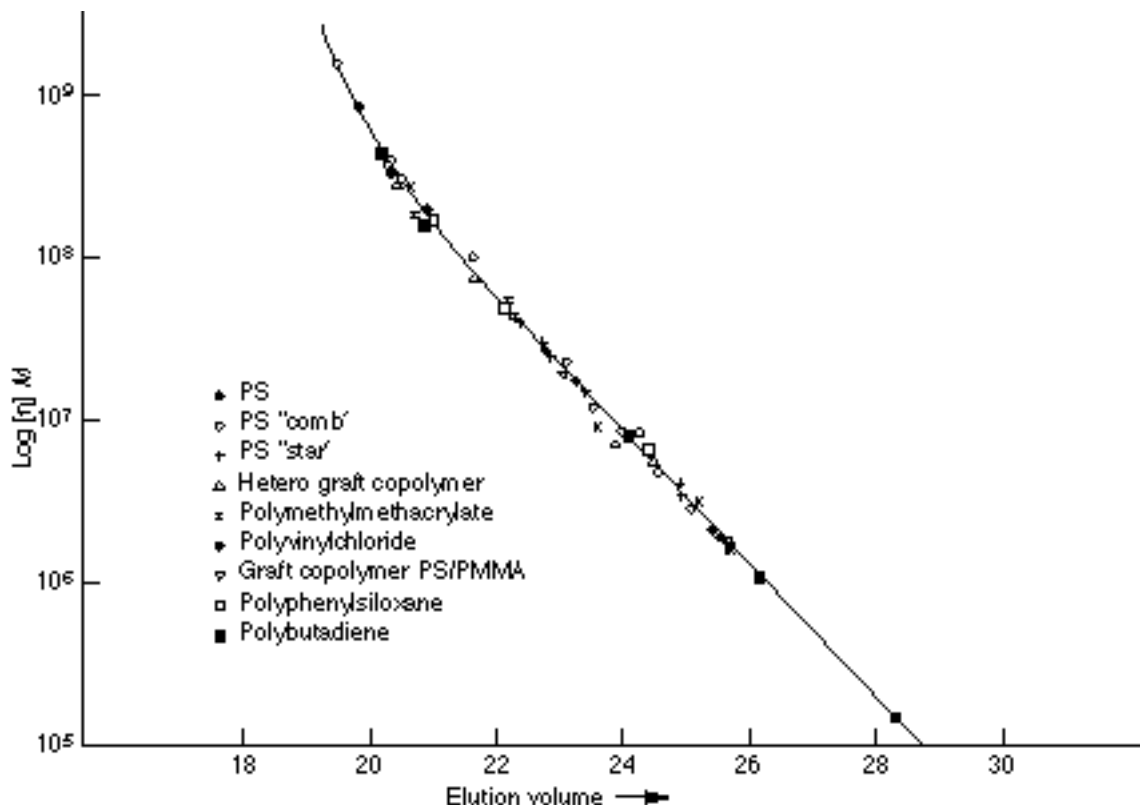
$$M_{i,A} = (k_B/k_A)^{1/(1+a_A)} M_{i,B}^R \quad (46.8)$$

where $R = (1 + a_B)/(1 + a_A)$.

Thus, with knowledge of the Mark-Houwink parameters for the standards and the sample (in the SEC eluent), one can determine absolute sample molecular weights. Published values of Mark-Houwink parameters in common SEC solvents are available for many polymers. When published constants are not available, they may be measured via Eq. (46.6). However, the measurement of k and a require standards of known molecular weight.

A third type of calibration in SEC is called broad-standard-calibration. Many types of broad-standard calibration schemes are used. The integral calibration scheme requires the complete MWD of a broad standard be known. Accurate characterization of a sample for the purposes of establishing it as a broad standard can be tedious and time-consuming. In many instances, a model such as the Flory most-probable model is used with the measured M_n or M_w to predict the complete MWD of a

Figure 46.4 Universal calibration plot. (From H. Benoit et al., *Journal of Polymer Science, Part B*, 5, 753, copyright © 1967. Reprinted by permission of John Wiley & Sons, Inc.)



broad standard. Use of this approach requires that the polymer standard closely follow the theoretical model, which limits the applicability of this approach.

A second, more widely used method for broad-standard calibration is the linear method. This approach requires a broad standard of known M_n and M_w . The method consists of an iterative search for the coefficients of a linear calibration equation ($\log M$ versus retention volume), such that the equation yields the known values of M_w and M_n for the broad standard.

Finally, one may determine the absolute calibration by using an absolute molar mass sensitive detector, such as a LALLS detector. In this approach, the absolute M_w is determined at each SEC elution volume increment. The plot of the determined $\log M_w$ versus retention volume represents the calibration curve. The calibration curve is then applied to the concentration detector chromatogram for determination of molecular weight averages and MWD.

Analytical Information

Qualitative

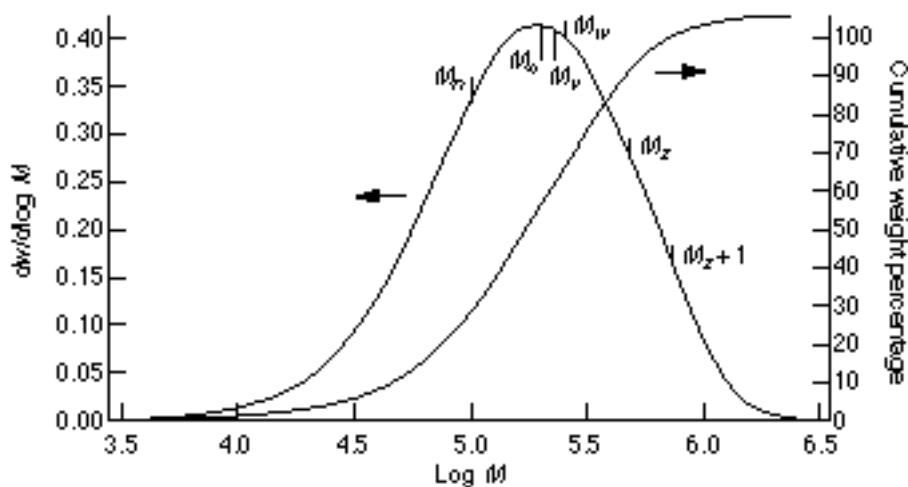
SEC is typically not used for qualitative analyses, as separation is governed by size, not composition. However, simple comparison of SEC chromatograms can provide qualitative information about differences in MWDs for unknowns having the same composition and topology.

Quantitative

SEC is used for determination of MWDs and molecular weight averages. MWDs are typically calculated and presented in one of two ways. The cumulative weight fraction MWD of a typical polystyrene sample is shown in Fig. 46.5. The right-hand y-axis is the cumulative weight percentage of chains having molecular weight less than M_i . A second, more popular, way of presenting MWD data is the differential weight (dw) fraction MWD, also shown for the same polystyrene sample in Fig. 46.5. Most commercially available SEC software packages provide these options for plotting MWD data. Consult the Suggested Readings section for more information regarding the details of converting a SEC chromatogram to a MWD plot.

Integrated SEC peak areas may also be used for determining the concentration of polymer in a matrix. For example, the concentration of polymer in a monomer sample may be determined via SEC. Typically, in these types of experiments, MWD information may not be desired. In these cases, a small pore size column is chosen such that the polymer is excluded (and thus well separated from the monomer peak). The excluded peak area is then used to calculate the concentration of polymer in the monomer sample. Determination of the concentration of polymer in other small-molecule matrices may be accomplished in a similar fashion.

Figure 46.5 Cumulative and differential weight fraction log MWDs for the same polystyrene sample. $M_p = 204685$, $M_n = 100988$, $M_w = 255949$, and $M_z = 482307$; polydispersity (M_w/M_n) = 2.534 with peak area = 906374.



Applications

The number of applications of SEC to the characterization of synthetic polymers and other macromolecules is enormous. The scope of this handbook does not allow for discussion of even a small fraction of the total SEC applications. A number of review articles have been written that adequately cover the recent applications of SEC to macromolecular characterization, and are cited in the Suggested Readings section. Two examples are presented here to give the reader a small sampling of typical SEC applications.

1. Determination of the MWD and Molecular Weight Averages of a Polystyrene Sample.

The absolute MWD of a polystyrene sample was determined by SEC using a peak position calibration curve generated from narrow-fraction polystyrene molecular weight standards. A solution of the polymer was prepared in tetrahydrofuran (THF) at a concentration of 2.0 mg/mL. The solution was filtered through a 0.2 μm filter before injection. Separation was performed on two mixed-gel SEC columns designed to provide a linear separation range for polystyrene from approximately 3×10^6 Da to 500 Da. THF at a flowrate of 1 mL/min was used as the eluent. A differential refractive index detector was used to detect the eluting peaks. A total of 17 narrow fraction polystyrene molecular weight standards were injected to determine the $\log M_w$ versus retention time calibration via the peak position method. The calibration curve was fit to a third-order polynomial as shown in Fig. 46.3. The resulting SEC chromatogram of the polystyrene sample is shown in Fig. 46.6, and the MWD is shown in Fig. 46.5 along with the calculated molecular weight averages.

Figure 46.6 SEC chromatogram of a polystyrene sample.

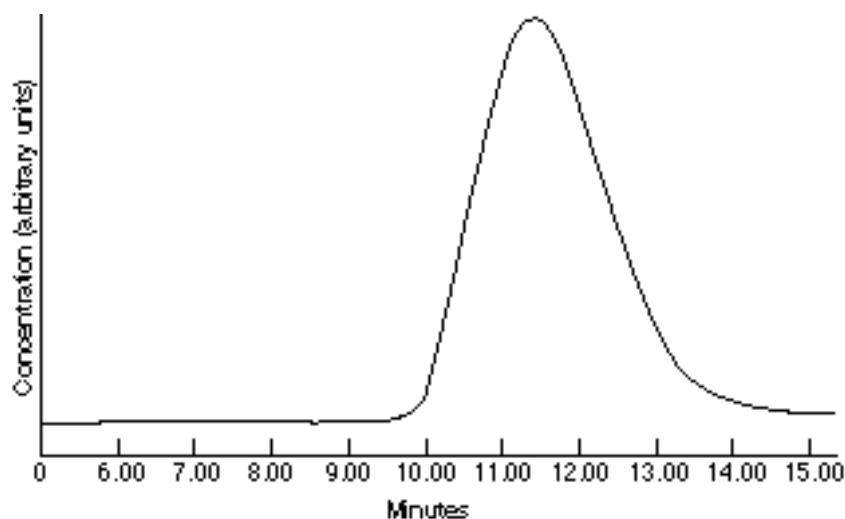
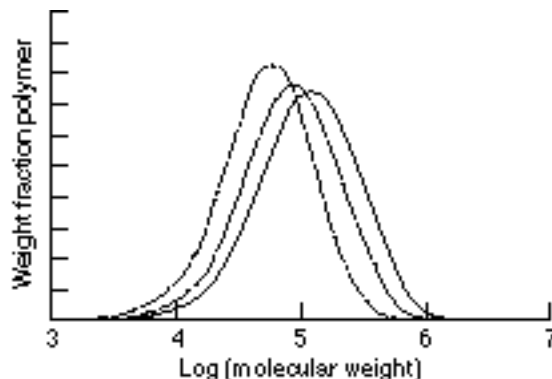


Figure 46.7 PEO apparent MWDs of polyurethane samples after various thermal processing steps. Virgin = solid curve, compounded = dashed curve, injection-molded = dot-dashed curve.



2. Comparison of the Apparent MWDs of Polyurethane Samples After Various Processing Steps.

Three polyurethane samples that had undergone various thermal cycles were analyzed by SEC. The samples were virgin pellets, compounded pellets, and an injection-molded part. SEC was performed on the same column set and detector described in the previous example. The eluent (and solvent for sample dissolution) was N,N-dimethylformamide containing 0.4% (w:v) lithium nitrate. Narrow-fraction polyethylene oxide (PEO) molecular weight standards were used for calibration. Thus, the results generated in this case are PEO apparent data. The resulting MWDs are overlaid in Fig. 46.7. Although the data are based on PEO standards, clearly there is a systematic decrease in molecular weight after each thermal processing step.

Nuts and Bolts

Relative Costs

Instruments for high-temperature SEC can be obtained for \$\$\$\$ to \$\$\$\$\$.

Pumps	\$\$
Columns	\$
Autosamplers	\$\$
Concentration detectors	\$ to \$\$
Data system	\$\$ to \$\$\$
Viscosity detector	\$\$\$

\$ = 1 to 5 K, \$\$ = 5 to 15 K, \$\$\$ = 15 to 50K, \$\$\$\$ = 50 to 100K, \$\$\$\$\$ = >100K.

Vendors for Instruments and Accessories

Alltech Associates (pumps, detectors, columns)
2051 Waukegan Rd.
Deerfield, IL 60015-1899
phone: 847-948-8600
fax: 847-948-1078
email: 73554.3372@compuserve.com
Internet: <http://www.alltechweb.com>

Hewlett-Packard (LC systems, data systems)
2850 Centerville Rd.
Wilmington, DE 19808
phone: 302-633-8504, 800-227-9770
fax: 302-633-8902
Internet: <http://www.hp.com/go/chem>

Micra Scientific Inc. (columns)
1955 Techny Rd., Suite 1
Northbrook, IL 60062
phone: 847-272-7877
fax: 847-272-7893
Internet: <http://www.micrasci.com>

Perkin-Elmer (LC systems, data systems)
761 Main Ave.
Norwalk, CT 06859-0001
phone: 800-762-4000
email: info@perkin-elmer.com
Internet: <http://www.perkin-elmer.com>

Polymer Laboratories (columns, detectors, high-temperature SEC instruments, data systems, molecular weight standards)
160 Old Farm Rd.
Amherst, MA 01002
phone: 413-253-9554
fax: 413-253-2476

TosoHaas (columns)
156 Keystone Dr.
Montgomeryville, PA 18936
phone: 215-283-9385, 800-366-4875
fax: 215-283-5035
Internet: <http://www.rohmhaas.com/tosohaas>

Varian Associates (LC systems, data systems, columns)
2700 Mitchell Dr.
Walnut Creek, CA 94598
phone: 510-939-2400, 800-926-3000
fax: 510-945-2102
Internet: <http://www.varian.com>

Waters Corp. (columns, high-temperature SEC instruments,
LC components, data systems)
34 Maple St.
Milford, MA 01757
phone: 508-478-2000, 800-252-4752
fax: 508-872-1990
email: info@waters.com
Internet: <http://www.waters.com>

Required Level of Training

The skill levels required for performing SEC experiments can vary greatly depending on the particular application. For example, a trained chemical technician can perform most routine SEC experiments where conditions for ideal SEC have been defined. On the other hand, development of ideal SEC conditions for complex polymer systems requires a far greater skill level. In these cases, training in analytical chemistry, separations science, and polymer chemistry and materials characterization is most useful.

Service and Maintenance

Service and maintenance requirements for SEC are no different from those required for conventional HPLC. Column performance can be evaluated on a daily basis through the calculation of DS (where D is the slope of the linear region of the SEC calibration curve and S is the peak variance for a monodisperse polymer peak), peak asymmetry, and number of theoretical plates.

Suggested Readings

- BARTH, H. G., AND B. E. BOYES, "Size Exclusion Chromatography," *Analytical Chemistry*, 64 (1992), 428R–42R.
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